CARBOHYDRATE RESEARCH

Carbohydrate Research 275 (1995) 371–379

The structure of the acidic exopolysaccharide produced by *Pseudomonas* "gingeri" strain Pf9

Paola Cescutti [a,b,*], Stanley F. Osman [a], William F. Fett [a], David Weisleder [c]

[a] *US Department of Agriculture, ARS, Eastern Regional Research Center, 600 East Mermaid Lane, Philadelphia, PA 19118, USA*

[b] *Dipartimento di Biochimica, Biofisica e Chimica delle Macromolecole, Università di Trieste, via L. Giorgieri 1, I-34127 Trieste, Italy*

[c] *US Department of Agriculture, ARS, National Center for Agricultural Utilization Research, 1815 North University Street, Peoria, IL 61604, USA*

Received 17 January 1995; accepted 29 April 1995

GENERAL INFORMATION

Languages

Papers will be published in English, French, or German.

Submission of manuscripts

Manuscripts should be sent to one of the Editors:

D.C. Baker, Department of Chemistry, The University of Tennessee, Knoxville, TN 37996-1600, USA.

J.G. Buchanan, School of Chemistry, University of Bath, Claverton Down, Bath BA2 7AY, UK.

J. Defaye, Laboratoire de Chimie des Glucides, DRFMC/SESAM/CENG, 17 rue des Martyrs, F-3805 Grenoble, Cédex 9, France.

D. Horton, Department of Chemistry, The American University, 4400 Massachusetts Ave., N.W., Washington, DC 20016-8014, USA.

E.F. Hounsell, Department of Biochemistry and Molecular Biology, University College London, Gower Street, London WC1E 6BT, UK.

J.P. Kamerling, Bijvoet Center for Biomolecular Research, P.O. Box 80.075, 3508 TB Utrecht, The Netherlands.

A.S. Serianni, Department of Chemistry and Biochemistry, University of Notre Dame, Stepan Chemistry Hall, Notre Dame, IN 46556, USA.

J.M. Webber, Crantock, 11 Little John's, Cross Hill, Exeter EX2 9PJ, UK.

Submission of an article is understood to imply that the article is original and unpublished and is not being considered for publication elsewhere.

Upon acceptance of an article by the journal, the author(s) will be asked to transfer the copyright of the article to the publisher. This transfer will ensure the widest possible dissemination of information.

Book reviews

Books suitable for review in the "Book reviews" section should be sent to E.F. Hounsell at the address given above.

Reprints

A total of 50 reprints of each paper will be provided free of charge to the author(s). Additional copies can be ordered at prices shown on the reprint order form which will accompany the proofs.

Subscriptions

Carbohydrate Research (ISSN 0008–6215). For 1995 volumes 263–279 are scheduled for publication in the period October 1994–December 1995.

Claims for issues not received should be made within six months of the publication (mailing) date of the issue. If not, they cannot be honored free of charge.

Subscriptions are accepted on a prepaid basis only and are entered on a calendar year basis.

Subscription information can be obtained from: Elsevier Science B.V., Journals Department, P.O. Box 211, 1000 AE Amsterdam, The Netherlands, Tel.: 31-20-4853642, FAX: 31-20-4853598, Telex: 18582.

Our p.p.h. (postage, packing and handling) charge includes surface delivery of all issues, except to subscribers in the USA, Canada, Australia, New Zealand, China, India, Israel, South Africa, Malaysia, Thailand, Singapore, South Korea, Taiwan, Pakistan, Hong Kong, Brazil, Argentina, and Mexico, who receive all issues by air delivery (SAL—Surface Air Lifted) at no extra cost. For Japan, air delivery requires 25% additional charge; for all other countries airmail and SAL charges are available upon request.

US mailing notice — Carbohydrate Research (ISSN 0008–6215) is published semi-monthly, total 34 issues, by Elsevier Science B.V., Molenwerf 1, P.O. Box 211, 1000 AE Amsterdam. Annual subscription price in the USA US $3239.00 (valid in North, Central, and South America only), including air speed delivery. Second class postage paid at Jamaica, NY 11431.

US POSTMASTERS: Send address changes to *Carbohydrate Research*, Publications Expediting, Inc., 200 Meacham Avenue, Elmont, NY 11003. Airfreight and mailing in the USA by Publications Expediting.

For further information, or a free sample copy of this or any other Elsevier Science journal, readers in the USA and Canada can contact the following address: Elsevier Science Inc., Journal Information Center, 655 Avenue of the Americas, New York, NY 10010, USA, Tel.: (212) 633 3750.

Information for Authors

See inside back cover

The structure of the acidic exopolysaccharide produced by *Pseudomonas* "gingeri" strain Pf9

Paola Cescutti [a,b,*], Stanley F. Osman [a], William F. Fett [a], David Weisleder [c]

[a] *US Department of Agriculture, ARS, Eastern Regional Research Center, 600 East Mermaid Lane, Philadelphia, PA 19118, USA*

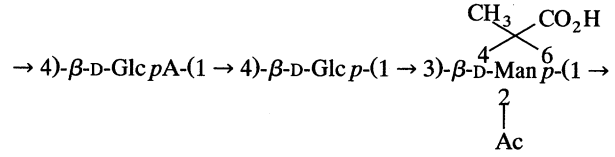
[b] *Dipartimento di Biochimica, Biofisica e Chimica delle Macromolecole, Università di Trieste, via L. Giorgieri 1, I-34127 Trieste, Italy*

[c] *US Department of Agriculture, ARS, National Center for Agricultural Utilization Research, 1815 North University Street, Peoria, IL 61604, USA*

Received 17 January 1995; accepted 29 April 1995

Abstract

The structure of the acidic exopolysaccharide produced by the mushroom pathogen *Pseudomonas* "gingeri" strain Pf9, a bacterium which causes ginger blotch, was investigated by chemical analysis, mass spectrometry and 1D and 2D NMR spectroscopy. The polysaccharide consists of the linear trisaccharide repeating unit



where the cyclic pyruvic acetal groups at O-4 and O-6 of the mannopyranosyl residues have the *S*-configuration. Methylation analysis under neutral conditions and NMR data showed that the mannose residues are acetylated at O-2. This exopolysaccharide has the same structure as the *E. coli* K55 capsular polysaccharide and differs from the *Klebsiella* K5 capsular polysaccharide only in the position of acetylation (C-2 of the glucopyranose residue).

Keywords: Pseudomonas "gingeri" strain Pf9; Mushroom pathogen; Acidic exopolysaccharide structure

* Corresponding author.

1. Introduction [1]

Pseudomonas "gingeri" is a mushroom (*Agaricus bisporus*) pathogen which causes a disease called ginger blotch characterized by the formation of ginger-coloured lesions on the mushroom caps. The disease was first reported to occur in the UK [1], later in Australia [2], and more recently in Pennsylvania, USA [3].

Eight of the ten strains of *P.* "gingeri" isolated by our group [3] exhibited a mucoid phenotype when grown on Difco *Pseudomonas* agar F at room temperature. Preliminary characterization of the "gingeri" EPS indicated the presence of glucose, mannose and glucuronic acid [4]. In this paper, we report the structure of the exopolysaccharide produced by one mucoid strain, Pf9, as part of our research program on the identification and structural elucidation of bacterial exopolysaccharides which may have various industrial and biotechnological uses.

2. Results and discussion

Isolation and composition of the Pf9 exopolysaccharide.—From 40 agar plates the strain yielded about 400 mg of crude EPS. The polysaccharide eluted as a single peak between 0.5 and 0.9 M NaCl on a DEAE-Sepharose column run under dissociative conditions. The neutral sugars identified by GLC of their aldononitrile or alditol acetate derivatives were glucose and mannose in a molar ratio of 1:2. The uronic acid was identified as glucuronic acid after carboxyl reduction of the EPS [5] followed by derivatization and GLC analysis. Methanolysis of the EPS followed by trimethylsilylation and subsequent GLC analysis showed the presence of glucuronic acid, glucose and mannose, together with a hexose substituted with a pyruvate group, as revealed by GLC–EI–MS and GLC–CI–MS (NH_4) analyses. All of the constituent sugars were shown to have the D-configuration by GLC analysis of their trimethylsilylated (−)-2-octyl glycosides [6]. The EPS contained 6.2% (w/w) of acetyl groups and 9.2% (w/w) of pyruvate groups, as determined by colorimetric [7] and enzymatic tests [8], respectively.

The ^1H NMR spectrum of the EPS showed two signals at high field (2.03 and 1.36 ppm), attributed to an acetyl and a pyruvate group, respectively. Integration indicated that these substituents are present in equimolar amounts. The ^{13}C NMR spectrum showed two signals at 25.4 and 21.0 ppm, which are consistent with the presence of a pyruvate methyl group in the *S*-configuration [9] and an acetyl group, respectively.

Methylation analysis and related experiments.—The native and depyruvated EPS were methylated and the derived alditol acetates were analysed by GLC–MS (Table 1). A sample of the depyruvated, methylated EPS was carboxyl-reduced and subsequently analysed with and without prior remethylation. GLC–MS of the derived alditol acetates gave the results shown in Table 1. These methylation analyses revealed that the EPS is

[1] Reference to brand or firm names does not constitute an endorsement by the US Department of Agriculture over others of a similar nature not mentioned.

Table 1
Methylation analysis of *Pseudomonas* "gingeri" EPS

Methylated sugar [a] (as alditol acetate)	t_R [b]	Molar ratio [c]			
		I	II	III	IV
2,4,6-Man	0.92		1.3	0.9	0.3
2,3,6-Glc	1.00	1.0	1.0	1.0	1.0 [e]
2,3-Glc [d]	1.17			0.8	
2-Man	1.22	0.9			

[a] 2,4,6-Man = 1,3,5-tri-*O*-acetyl-2,4,6-tri-*O*-methylmannitol, etc.
[b] Retention time relative to that of 2,3,6-Glc.
[c] I, methylated EPS; II, depyruvated methylated EPS; III, depyruvated–methylated then carboxyl-reduced EPS; IV, depyruvated–methylated then carboxyl-reduced and remethylated EPS.
[d] C-6 dideuterated according to GLC–MS.
[e] 50% of 2,3,6-Glc is C-6 dideuterated, as revealed by the presence of equal amounts of the primary fragments at m/z 233 and 235 and of the secondary fragments at m/z 173 and 175 in the GC mass spectrum.

composed of 4-linked glucuronic acid, 4-linked glucose, and 3-linked mannose. The pyruvic acid is linked to O-4 and O-6 (acetal form) of the mannose residue.

The β-elimination reaction was performed on the native methylated EPS followed by realkylation with CD_3I. GLC–MS of the alditol acetate derivatives indicated the presence of a new component, namely a terminal glucose substituted with a CD_3 group on O-4, thus revealing that the glucuronic acid is linked to the glucose residue. The greater stability of the glycosiduronic linkage explains the uneven molar ratio of the glucose and mannose residues found in the neutral sugar analysis.

In order to determine the position of acetyl substitution, a sample of depyruvated EPS was methylated under neutral conditions [10] and the derived alditol acetates were analysed by GLC–MS. A new derivative, indicative of a 2,3-linked mannose, was present together with 4-linked glucose and a small amount of 3-linked mannose, indicating that the O-2 of the mannopyranosyl residue bears the acetyl group.

NMR studies of the deacetylated–depyruvated exopolysaccharide.—The ^1H NMR spectrum (not shown) showed three anomeric signals in the region 4.80–4.50 ppm, suggesting a trisaccharide repeating unit. The sugars giving rise to these ^1H resonances were named **a** to **c** in order of decreasing chemical shift. The **b** anomeric signal had a very small coupling constant (< 3 Hz), typical of hexopyranosides in the *manno* configuration, while the **a** and **c** signals had $^3J_{H1,H2}$ values of 7.4 and 7.6 Hz, respectively, indicating two β-anomers.

The ^{13}C NMR spectrum contained C-1 signals at 103.78, 101.36, and 101.15 ppm and a total of 18 signals, thus confirming the presence of a trisaccharide repeating unit. The $^1J_{C1,H1}$ values obtained from a coupled ^{13}C experiment were characteristic of three β-linkages ($161.5 < J_{C1,H1} < 166.0$) [11]. Furthermore, the carboxyl resonance at 176.50 ppm confirmed the presence of a uronic acid residue.

Complete assignment of the ^1H resonances was obtained from ^1H–^1H correlation (COSY [12], RELAY COSY [13], TOCSY [14]) experiments, while the corresponding ^{13}C chemical shifts were deduced from ^1H–^{13}C correlation (HETCOR [15] and HMBC [16,17]) experiments (Fig. 1). The chemical shifts for each spin system are reported in

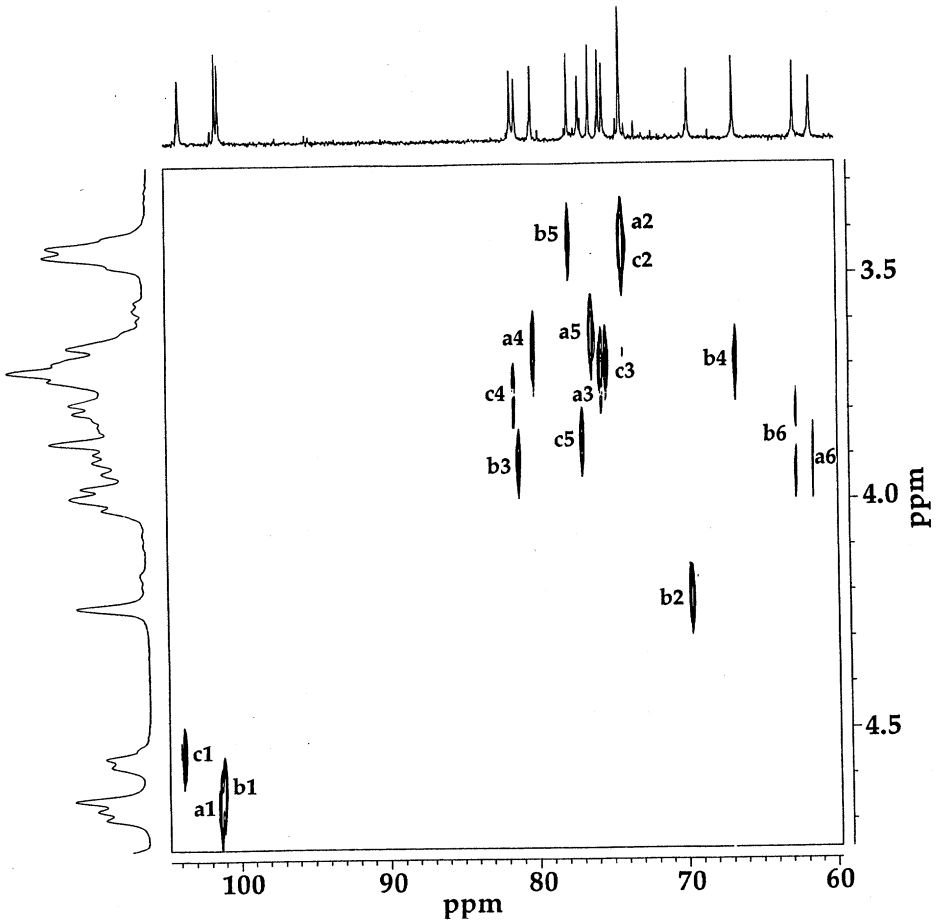


Fig. 1. $^{1}H-^{13}C$ correlation map of the deacetylated–depyruvated EPS. **a1** indicates the cross-peak between H-1 and C-1 of residue **a**, etc.

Table 2. On the basis of its small $^{3}J_{H1,H2}$, spin system **b** was assigned to the mannopyranosyl residue; it followed that **a** is the glucopyranosyl residue and **c** the glucopyranosiduronic acid residue. The assignments for the three spin systems are in good agreement with literature data [18–20].

NMR studies of the depyruvated exopolysaccharide.—The anomeric region of the ^{1}H NMR spectrum contained two new resonances at 5.57 and 4.85 ppm together with a group of signals in the interval 4.68–4.39 ppm which were not resolved. A high-field singlet at 2.21 ppm indicated the presence of an *O*-acetyl group in the approximate amount of one every two repeating units, thus suggesting that about 50% of the substituent was lost in the acidic removal of the pyruvate groups.

The ^{13}C NMR spectrum was complicated, with five signals in the region 103.89–99.91 ppm; comparison with the ^{13}C NMR spectrum of the deacetylated–depyruvated EPS

Table 2
^{1}H and ^{13}C data for the deacetylated–depyruvated EPS at 27°C

Residue		^{1}H or ^{13}C chemical shifts (ppm) [a]						
		1	2	3	4	5	6	6′
a	^{1}H	4.68	3.43	3.71	3.67	3.63	3.84	4.01
4)-β-Glc	^{13}C	101.36	74.24	75.67 [b]	80.19	76.3	61.46	
b	^{1}H	4.64	4.22	3.92	3.71	3.46	3.96	3.78
3)-β-Man	^{13}C	101.15	69.64	81.28	66.60	77.77	62.57	
c	^{1}H	4.56	3.46	3.70	3.78	3.88		
4)-β-GlcA	^{13}C	103.78	74.24	75.40 [b]	81.56	77.02	176.5	

[a] Relative to internal acetone at 2.23 ppm and 31.07 ppm for ^{1}H and ^{13}C, respectively; 1–6′ are atom numbers.
[b] Assignments may be reversed.

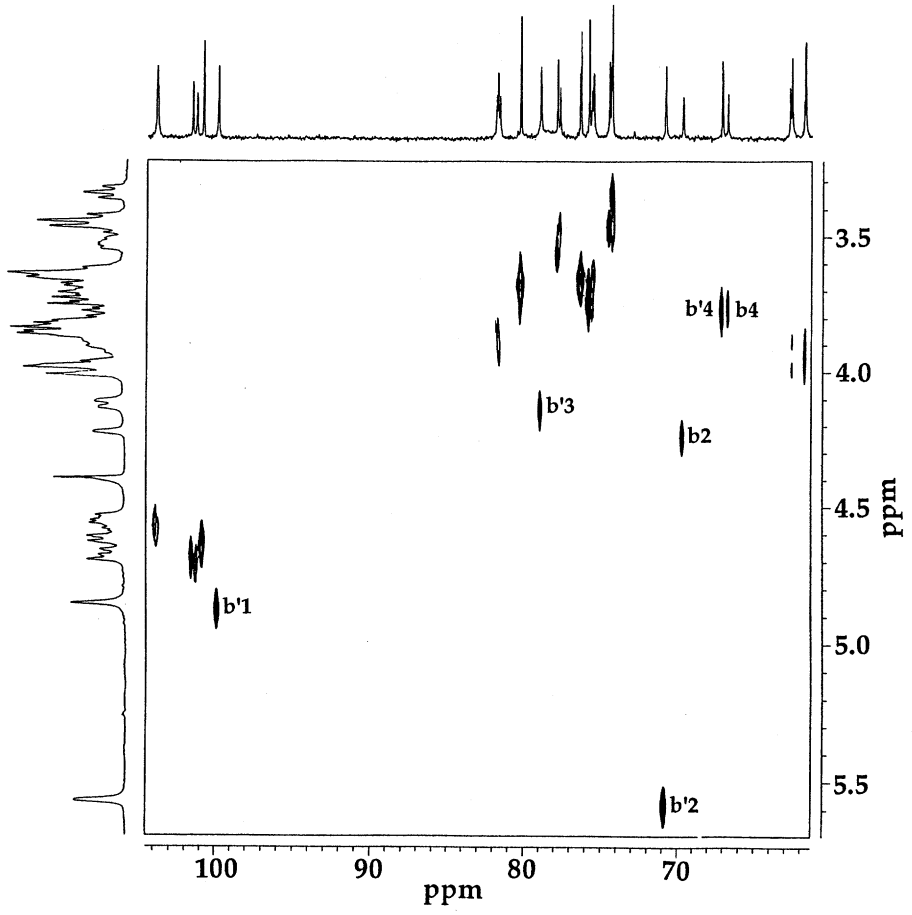


Fig. 2. ^{1}H–^{13}C correlation map of the depyruvated EPS. Some of the C–H correlations are shown: **b** and **b′** indicate the non-acetylated and the acetylated mannose residues, respectively.

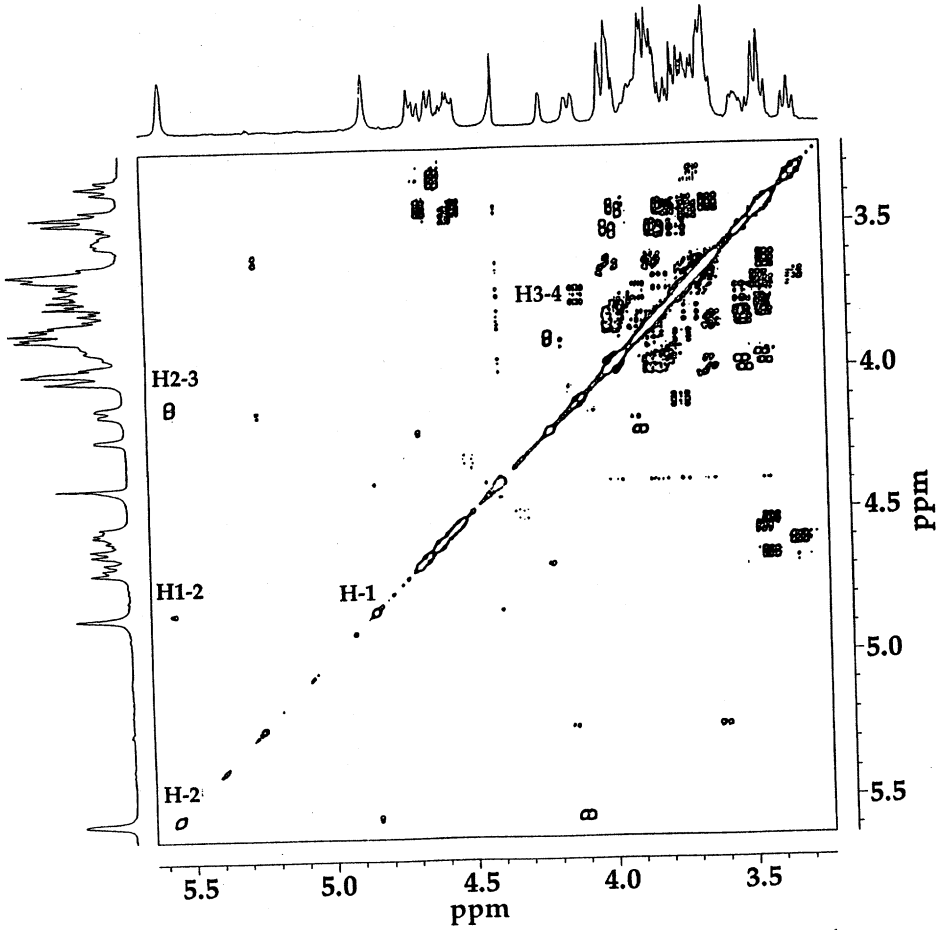


Fig. 3. COSY contour plot of the region 5.65–3.20 ppm for the depyruvated EPS. Some of the ^{1}H resonances for the acetylated mannose residue are shown.

revealed the presence of new resonances also in the ring region. Inspection of the HETCOR diagram (Fig. 2) showed that the signals at 5.57 and 4.85 ppm were connected to carbons not present in the ^{13}C NMR spectrum of the deacetylated–depyruvated EPS and resonating at 70.89 and 99.91 ppm, respectively. The signal at 5.57 ppm was assigned to the ring proton geminal to the attached *O*-acetyl group [21]. This signal shifted into the anomeric region as a result of the *O*-acetylation chemical shift effect. Because of its very small $^{3}J_{H1,H2}$ the new anomeric signal at 4.85 ppm was ascribed to H-1 of a mannose residue. In the COSY plot (Fig. 3) the anomeric signal at 4.85 ppm connected in sequence to the resonances at 5.57 ppm (H-2), 4.12 ppm (H-3) and 3.74 ppm (H-4), thus showing that the mannose residue is the one substituted with an acetyl group on O-2. The chemical shifts of the ^{13}C resonances for the acetylated mannose residue were obtained from inspection of the HETCOR plot (C-1 to C-4) and of the ^{13}C

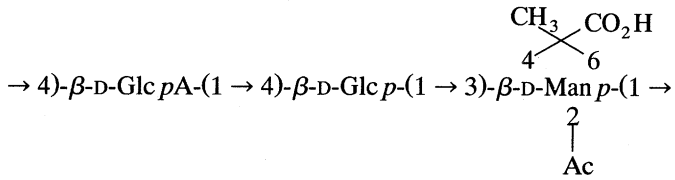
Table 3
^{1}H and ^{13}C data for the acetylated mannose residue at 60°C

Residue		^{1}H or ^{13}C chemical shifts (ppm) [a]						
		1	2	3	4	5	6	6′
	^{1}H	4.87	5.57	4.14	3.74	3.46	4.02	3.84
3)-β-Man	^{13}C	99.91	70.89	79.00	67.15	77.92	62.68	

[a] Relative to internal acetone at 2.23 ppm and 31.07 ppm for ^{1}H and ^{13}C, respectively; 1–6′ are atom numbers.

NMR spectrum (C-5 and C-6). The ^{1}H and ^{13}C NMR data for the acetylated mannose residue are given in Table 3. The effects of *O*-acetylation on the chemical shifts of the ^{1}H resonances are consistent with literature data [21,22]. In fact, the α effect (i.e., the effect on the proton attached to the carbon atom substituted with the *O*-acetyl group) observed is equal to 1.35 ppm, while the β effect (i.e., the effect on the protons attached to the carbon atoms next to the *O*-acetylated carbon) is 0.23 ppm for H-1 and 0.22 ppm for H-3. The shifts observed for the ring carbon resonances upon acetylation are also in good agreement with previous data [22]. The substituted carbon (C-2) is shifted downfield by 1.25 ppm, while the adjacent C-1 and C-3 are shifted upfield (1.24 and 2.28 ppm, respectively).

Based on the data presented in this report, the structure of the Pf9 exopolysaccharide is



This structure is identical with that of *E. coli* K55 [23] capsular polysaccharide and very similar to that of *Klebsiella* K5 [24] capsular polysaccharide. It is interesting that *Klebsiella* and *E. coli* belong to the enterobacteriacae family while *P.* "gingeri" belongs to the Pseudomonas family and is a mushroom pathogen. However, the production of the same polysaccharide by these three different microorganisms may indicate a common role in the pathogen process.

3. Experimental

General methods.—Analytical GLC of the aldononitrile and alditol acetates and partially methylated alditol acetate derivatives was performed with a Hewlett–Packard Model 5890 gas chromatograph equipped with a flame ionization detector and an SP2330 capillary column (15 m) (Supelco), using He as the carrier gas. The following temperature programme were used: for alditol acetates and aldononitrile acetates, 150 to 250°C at 4°C/min; for methylated alditol acetates, 125 to 250°C at 4°C/min. The trimethylsilylated octyl and methyl glycosides were analysed on an HP5 column (30 m)

(Hewlett–Packard) using the same temperature programme as for the alditol acetate derivatives. GLC–MS was performed on a Hewlett–Packard model 5995B instrument using the same columns and temperature programme.

Pseudomonas "gingeri" strain Pf9 was grown on Difco *Pseudomonas* agar F (PAF) contained in culture dishes (100 × 15 mm) for 2–3 days at room temperature and the polysaccharide was isolated as described previously [4].

The EPS was methanolysed with 2 M HCl in MeOH for 24 h at 75°C. Hydrolyses were carried out with 2 M CF_3CO_2H at 120°C for 1 h. Alditol acetates were prepared as described previously [25]. Aldononitrile acetates were prepared according to Varma et al. [26].

Anion-exchange chromatography of the EPS was performed on a DEAE-Sepharose column with 0.05 M Tris–HCl buffer (pH 6.5) containing 7 M urea [27]. A salt gradient (0–2 M NaCl) in the same buffer was used as the eluent.

Methylation analysis and related experiments.—Methylations were performed according to the modified Hakomori [28] method using potassium methylsulfinylmethanide [29]. The methyl-esterified carboxyl groups were reduced with $LiAlD_4$ [30]. β-Elimination reaction of the methylated polysaccharide was carried out using a modification [31] of the method of Lindberg et al. [32]. The partially methylated alditol acetates obtained from these reactions were analysed by GLC–MS.

Removal of the non-carbohydrate substituents.—The pyruvic acid substituent was hydrolysed [33] with 0.5 M oxalic acid at 100°C for 90 min. The *O*-acetyl groups were removed [34] by treatment with 0.01 M NaOH at room temperature for 5 h.

NMR spectroscopy.—The sample was dissolved in 99.96% deuterium oxide containing a trace of acetone as internal reference (2.23 ppm for ^1H and 31.07 ppm for ^{13}C). Spectra were recorded at 300 K for the deacetylated–depyruvated EPS and at 330 K for the depyruvated EPS on a Bruker ARX-400 spectrometer, equipped with a 5 mm $^{13}C/^1H$ dual probe. Standard Bruker pulse programs (UXNMR 930601.5) were used.

Acknowledgements

We thank Dr R. Dudley and Ms J. Brouillette for recording NMR spectra and Dr P. Pfeffer for helpful discussions.

References

[1] W.C. Wong, J.T. Fletcher, B.A. Unsworth, and T.F. Preece, *J. Appl. Bacteriol.*, 52 (1982) 43–48.

[2] S.S. Cutri, B.J. Macauley, and W.P. Roberts, *J. Appl. Bacteriol.*, 57 (1984) 291–298.

[3] J.M. Wells, G.M. Sapers, W.F. Fett, J.E. Butterfield, J.B. Jones, H. Bouzar, and F.C. Miller, *Phytopathology*, submitted for publication.

[4] W.F. Fett, J.M. Wells, C. Wijey, and P. Cescutti, *Appl. Environ. Microbiol.*, submitted for publication.

[5] R.L. Taylor and H.E. Conrad, *Biochemistry*, 11 (1972) 1383–1388.

[6] K. Leontein, B. Lindberg, and J. Lönngren, *Carbohydr. Res.*, 62 (1978) 359–362.

[7] E.A. McComb and R.M. McCready, *Anal. Chem.*, 29 (1957) 819–821.

[8] A. Jeanes, P. Rogovin, M.C. Cadmus, R.W. Silman, and C.A. Knutson, *ARS Bulletin NC-51*, US Department of Agriculture, Washington, DC, 1976.
[9] P.J. Garegg, P-E. Jansson, B. Lindberg, F. Lindh, J. Lönngren, I. Kvarnström, and W. Nimmich, *Carbohydr. Res.*, 78 (1980) 127–132.
[10] P. Prehm, *Carbohydr. Res.*, 78 (1980) 372–374.
[11] K. Bock, I. Lundt, and C. Pedersen, *Tetrahedron Lett.*, 13 (1973) 1037–1040.
[12] A. Bax and R. Freeman, *J. Magn. Reson.*, 44 (1981) 542–561.
[13] A. Bax and G. Drobny, *J. Magn. Reson.*, 61 (1985) 306–320.
[14] A. Bax and D.G. Davis, *J. Magn. Reson.*, 65 (1985) 355–360.
[15] A. Bax and D.G. Davis, *J. Magn. Reson.*, 42 (1981) 501–505.
[16] L. Lerner and A. Bax, *Carbohydr. Res.*, 166 (1987) 35–46.
[17] A. Bax and M.F. Summers, *J. Am. Chem. Soc.*, 108 (1986) 2093–2094.
[18] K. Bock, C. Pedersen, and H. Pedersen, *Adv. Carbohydr. Chem. Biochem.*, 42 (1984) 193–225.
[19] J.H. Bradbury and G.A. Jenkins, *Carbohydr. Res.*, 126 (1984) 125–156.
[20] M. Bartelt, A.S. Shashkov, H. Kochanowski, B. Jann, and K. Jann, *Carbohydr. Res.*, 254 (1994) 203–212.
[21] J. Haverkamp, H. Van Halbeek, L. Dorland, J.F.G. Vliegenthart, R. Pfeil, and R. Schauer, *Eur. J. Biochem.*, 122 (1982) 305–311.
[22] P. Cescutti, N. Ravenscroft, S. Ng, Z. Lam, and G.G.S. Dutton, *Carbohydr. Res.*, 244 (1993) 325–340.
[23] A.N. Anderson and H. Parolis, *Carbohydr. Res.*, 188 (1989) 157–168.
[24] G.G.S. Dutton and M. Yang, *Can. J. Chem.*, 51 (1973) 1826–1832.
[25] P. Albersheim, D.J. Nevins, P.D. English, and A. Karr, *Carbohydr. Res.*, 5 (1967) 340–345.
[26] R. Varma, R.S. Varma, and A.H. Wardi, *J. Chromatogr.*, 77 (1973) 222–227.
[27] G. Orgambide, H. Montrozier, P. Servin, J. Roussel, D. Trigalet-Demery, and A. Trigalet, *J. Biol. Chem.*, 266 (1991) 8312–8321.
[28] S. Hakomori, *J. Biochem.*, 55 (1964) 205–208.
[29] L.R. Phillips and B.A. Fraser, *Carbohydr. Res.*, 90 (1981) 149–152.
[30] P. Aman, L.-E. Franzén, J.E. Darvill, M. McNeil, A.G. Darvill, and P. Albersheim, *Carbohydr. Res.*, 103 (1982) 77–100.
[31] G.O. Aspinall and K.-G. Rosell, *Carbohydr. Res.*, 57 (1977) c23–c26.
[32] B. Lindberg, J. Lönngren, and J.L. Thompson, *Carbohydr. Res.*, 28 (1973) 351–357.
[33] S.F. Osman and W.F. Fett, *J. Bacteriol.*, 171 (1989) 1760–1762.
[34] A. Amemura, T. Harada, M. Abe, and S. Higashi, *Carbohydr. Res.*, 115 (1983) 165–174.